Pestic. Sci. 1998, **52**, 275–281

Behaviour of Fipronil in Soil under Sahelian Plain Field Conditions

Alain Bobé,[1] Jean-François Cooper,[1]* Camille M. Coste[2] & Marie-Anne Muller[3]

[1] Laboratoire de Chimie Analytique, Faculté de Pharmacie, av. Charles Flahault, 34060 Montpellier, France
[2] Centre de Phytopharmacie, av. de Villeneuve, 66000 Perpignan, France
[3] Secteur Résidus Service Analyse Rhône-Poulenc Agro, 14/20 rue Pierre Baizet, 69009 Lyon, France

(Received 4 December 1996; revised version received 22 April 1997; accepted 28 October 1997

Abstract: The behaviour of fipronil, a phenylpyrazole insecticide used for locust control, was studied under sub-Saharan conditions in soils of the Niamey region of Niger. A formulation of fipronil (Adonis®) was applied to uncultivated soils at Banizoumbou and Saguia. Soil was sampled at 0–10, 10–20 and 20–30 cm depths for up to two months after treatment. Residues were analysed by gas chromatography using electron capture and mass detectors. For both soils, a rapid initial decrease of fipronil was observed, with rapid formation for the most part of a photodegradate. Three other metabolites of fipronil were also detected throughout the study. These metabolites displayed different dissipation kinetics. Fipronil and its metabolites did not move beyond 10 cm depth, except for the amide, which is not considered a toxicologically significant metabolite. © 1998 SCI.

Pestic. Sci., **52**, 275–281 (1998)

Key words: fipronil; locust control; tropical conditions

1 INTRODUCTION

Fipronil, (±)-5-amino-1-(2,6-dichloro-α,α,α-trifluoro-*p*-tolyl)-4-trifluoromethylsulfinylpyrazole-3-carbonitrile, a recently developed phenylpyrazole insecticide (Rhône-Poulenc Agro) is used notably in locust control. It is active against locusts at low rates of use (2–8 g ha^{-1}) through both contact action and ingestion. Fipronil has been shown to interfere with the passage of chloride ions through the gamma-aminobutyric acid (GABA)-regulated chloride channel, thereby disrupting central nervous system activity and, at sufficient concentrations, causing death.[1–4] Its wide range of efficacy (locusts and grasshoppers, eggs at the point of hatching, nymphs and adults) and treatment methods (total cover or barrier treatment, preventive or curative control) in agricultural, forestry or pastoral situations give it an advantage over the growth regulators used in the chemical control of locusts.[5]

Few data are available on the environmental degradation of fipronil under tropical conditions, especially in the Sahelian environment which is particularly prone to locust invasions. In order to study the behaviour of the active ingredient under field conditions, we joined two teams (Agrhymet Crop Protection Training Department and Cirad-Gerdat-Prifas) working locally on the efficacy of the product. This study was carried out in Niger in September 1995 to evaluate the possible movement of fipronil in soil and the formation and fate of its major metabolites, which were identified following studies carried out under temperate plain field conditions (Rhône-Poulenc Agro, 1995, pers. comm.) and confirmed in our laboratories.

2 EXPERIMENTAL SECTION

2.1 Characteristics of working areas

Two plots of 1600 m^2 and 1215 m^2 were chosen at Banizoumbou (75 km east of Niamey) and Saguia

* To whom correspondence should be addressed.

 Pestic. Sci. 0031-613X/98/$17.50. Printed in Great Britain

TABLE 1
Characteristics of Soils Used

Characteristics	*Banizoumbou*			*Saguia*		
Layer	*0–10 cm*	*10–20 cm*	*20–30 cm*	*0–10 cm*	*10–20 cm*	*20–30 cm*
Clay (%)	2·4	9·6	5·2	0·6	2·4	2·2
Fine silt (%)	1·8	2·1	0·3	0·6	0·3	0·1
Coarse silt (%)	16·4	4·6	6·7	0·8	1·3	1
Fine sand (%)	25·6	49·6	46	47·3	45	52·1
Coarse sand (%)	53·8	34	41·7	50·7	50·9	44·7
Org. mat. (%)	0·26	0·28	0·22	0·09	0·1	0·14
Org. carbon (%)	0·15	0·16	0·13	0·05	0·06	0·08
CEC (meq/100 g)	0·74	1·05	0·85	0·72	0·98	1·07
pH (H_2O)	5·8	4·7	4·15	5·3	5·25	5·25

(10 km to the south). These plots had never been treated with fipronil and were manually weeded before treatment. Soil properties are summarised in Table 1. These two soils were similar, except for higher silt levels in the surface layer at Banizoumbou. Meteorological data were recorded daily throughout the experiment. Average maximum temperatures were 37.5 (±2·5)°C at Saguia, and 38·3 (±2·3)°C at Banizoumbou, showing little difference throughout the course of the study. As shown in Fig. 1, heavy rains (64 mm) occurred at Banizoumbou on the day after treatment, whilst at Saguia, no rainfall occurred after application of fipronil. Hours of sunshine are also indicated in Fig. 1. On the day of treatment, Banizoumbou received slightly less sunshine than Saguia (5.6 and 8 h respectively).

2.2 Treatment and sampling

Each plot was treated with 2 litres of fipronil 4 g litre^{-1} UL ('Adonis'® 4-UL), corresponding to 8 g AI ha^{-1}, double the dose recommended for such a treatment. This was applied using a battery-powered rotary sprayer (micro-ULVA; Micron Sprayers, Bromyard UK). Sampling of soil was done using a methodology based on that of FAO.[6] A composite sample was made, consisting of several sub-samples taken at various points along the diagonals of rectangular plots. Samples of 300 to 500 g of soil from the same layer were taken with a corer according to the following schedule: 0 (or 0·5 for Saguia), 1, 3, 8, 14, 25 (or 28) and 57 (or 53) days after treatment at 0–10, 10–20 and 20–30 cm depth. The

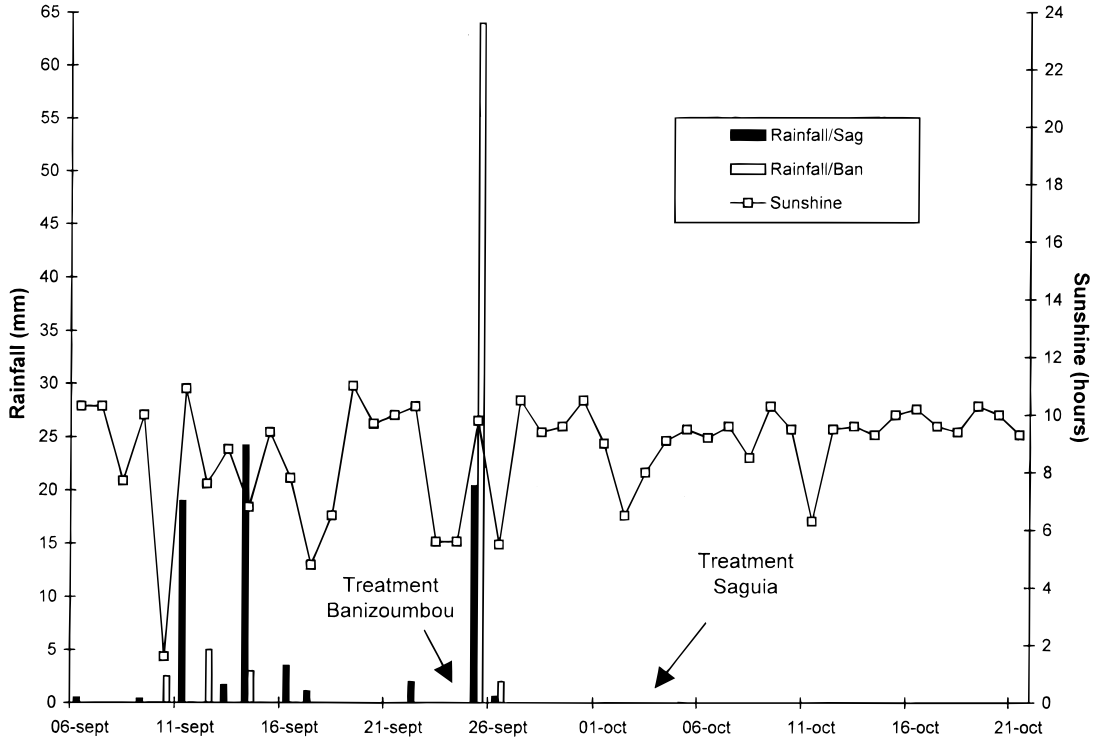

Fig. 1. Rainfall and sunshine in Banizoumbou and Saguia during the first month after treatment (from: Agrhymet and Orstom; Niamey-Niger).

corer was made in the laboratory to enable the sampling of the different layers of soil with minimum risk of contamination of the lower levels with soil from above. It consisted of three metallic cylinders fitting one inside the other, the smallest diameter cylinder taking soil from the deepest soil layer. Untreated soil samples were taken before treatment. Samples were stored at −22°C until analysis. Soil water content was determined for all samples. The (relatively low) values ranged from 1–2% and from 1–7% for the upper soil layers in Saguia and Banizoumbou respectively.

2.3 Analytical methodology

The analytical procedure employed was adapted from an unpublished method developed by Rhône-Poulenc Agro.

2.3.1 Reagents and materials

(a) All chemicals and solvents used were analytical grade or pesticide analysis grade. Analytical grade fipronil, four of its metabolites (listed in Table 2) and 'Adonis'® 4 UL were obtained from Rhône-Poulenc Agro, Lyon, France.
(b) SPE Cartridges silica Lichrolut® Si 500 mg, 3 ml.
(c) Glass microfibre filters Whatman GF/B.

2.3.2 Apparatus

(a) Blender: 'Omni-mixer' type 'Coupatan-R' with 300-ml glass vessels.
(b) Rotary evaporator, type Rotavapor Buchi-EL.
(c) Gas liquid chromatographs (GLC):

—GC Hewlett-Packard HP 5890 connected to a mass spectrometric detector HP MSD 5971A. Electron impact mode 70 eV. Splitless injection mode (injection purge off: 0·75 min). Injector temperature 250°C. Transfer line temperature 280°C. Column: SPB-17; 30 m; 0·25 mm ID; 0·25 μm film thickness. Initial oven temperature 70°C for 1 min + 50°C min^{-1} up to 240°C for 16 min. Solvent delay 3 min. Carrier gas helium 5·5 (purity 99·9995%) at a flow rate of 1·2 ml min^{-1}. For Single Ion Monitoring (SIM), five groups of ions were chosen: fipronil (351; 367 uma), metabolite A (333; 388 uma), metabolite B (351; 420 uma), metabolite C (383; 452 uma) and metabolite D (255; 385 uma).

—GC Hewlett-Packard HP 5890 equipped with a ^{63}Ni electron-capture detector (ECD). Injector temperature 280°C. Detector temperature 300°C. Column: J & W Scientific DB-1701; 30 m; 0·32 mm ID; 0·25 μm film thickness. Initial oven temperature: 70°C for 1 min + 50°C min^{-1} up to 240°C for 16 min. Carrier gas helium 4·5 (purity 99·995%) at a flow rate of 1·8 ml min^{-1} with argon/methane 90/10 as auxiliary gas (50 ml min^{-1}). Data treatment software Hewlett-Packard HP 3365 Series II Chemstation (DOS Series).

2.3.3 Analysis and quality parameters

2.3.3.1 Extraction. Air-dried soil samples (50 g in duplicate) were extracted by mixing for 30 and 20 min with acetonitrile + acetone (70 + 30 by volume; 2 × 80 ml). The extracts were combined and dried by passing through anhydrous sodium sulfate on a glass microfibre filter. The filtrate was evaporated just to dryness and the residue taken up with petroleum ether (b.p. 35–60°C; 2 ml) prior to the clean-up step performed on a Lichrolut® silica cartridge.

2.3.3.2 Clean-up. After conditioning a silica cartridge with petroleum ether + acetone (70 + 30 by volume; 2 ml) and petroleum ether (2 ml), the extract was placed on the cartridge, which was then eluted with petroleum ether + acetone (70 + 30 by volume; 10 ml). This eluate was evaporated to dryness and the residue taken up into toluene (0·5 or 1 ml).

2.3.3.3 Instrumental analysis. Each extract was injected into a GC column equipped with an electron-capture detector and/or a mass detector to confirm positive results. An ECD chromatogram of an extract of treated soil sample (day 3, Banizoumbou) is shown in Fig. 2a. Confirmation of the result is presented in Fig. 2b (SIM-

TABLE 2
Fipronil and Its Metabolites

Common name	*Chemical name (IUPAC)*	*Molecular weight*
Fipronil	(±)-5-amino-1-(2,6-dichloro-α,α,α-trifluoro-*p*-tolyl)-4-trifluoromethylsulfinylpyrazole-3-carbonitrile	437·14
Metabolite A	5-amino-1-(2,6-dichloro-α,α,α-trifluoro-*p*-tolyl)-4-trifluoromethylpyrazole-3-carbonitrile	389·09
Metabolite B	5-amino-1-(2,6-dichloro-α,α,α-trifluoro-*p*-tolyl)-4-trifluoromethylthiopyrazole-3-carbonitrile	421·10
Metabolite C	5-amino-1-(2,6-dichloro-α,α,α-trifluoro-*p*-tolyl)-4-trifluoromethylsulfonylpyrazole-3-carbonitrile	453·10
Metabolite D	(±)-5-amino-1-(2,6-dichloro-α,α,α-trifluoro-*p*-tolyl)-4-trifluoromethylsulfinylpyrazole-3-carboxamide	455·08

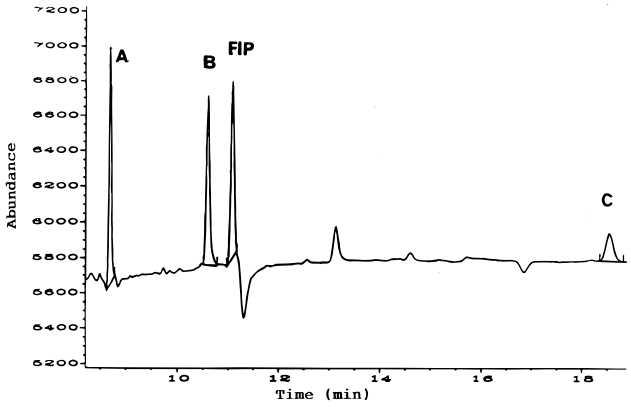

Fig. 2a. GC/ECD chromatogram of an extract of a treated soil sample (day 3, Banizoumbou).

TIC or Total Ion Chromatogram) and Fig. 2c (SIM-Single Ion Chromatograms, allowing individual determination of fipronil and metabolite B in spite of their similar retention times).

2.3.3.4 Recovery studies. To determine method efficiency, untreated Banizoumbou and Saguia soil samples were fortified with known amounts (0·2, 0·05 and 0·005 mg kg^{-1}) of analytical standards dissolved in toluene. Each sample, at three levels of fortification, was analysed in duplicate. The mean recoveries were 85(±5)% for fipronil, 93(±8)% for metabolite A, 87(±5)% for B, 96(±10)% for D and 100(±5)% for C.

2.3.3.5 Limit of quantification (LOQ). The limit of quantification was defined as the sample concentration

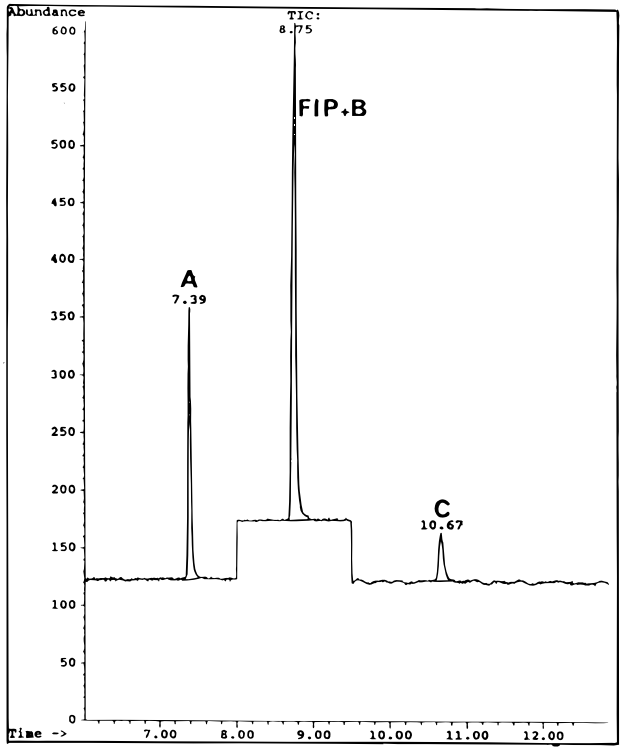

Fig. 2b. GC/MS chromatogram (SIM-Total Ion Chromatogram) of an extract of a treated soil sample (day 3, Banizoumbou).

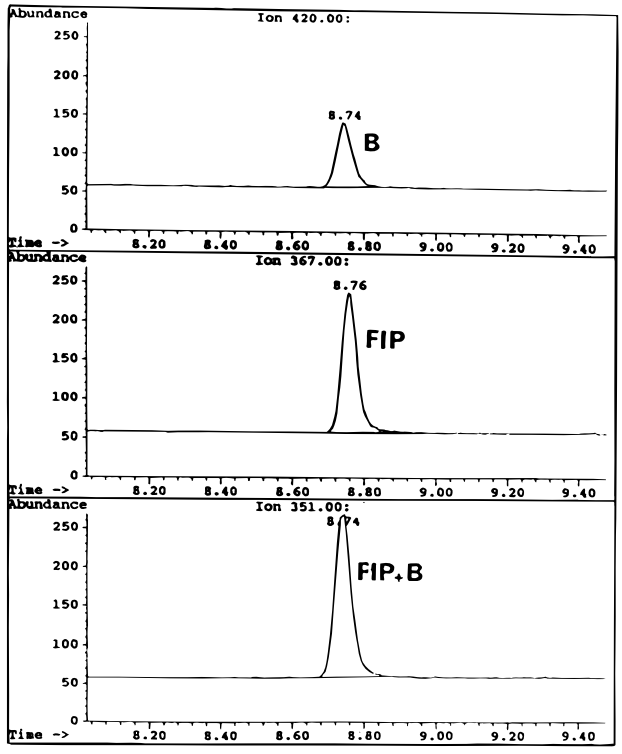

Fig. 2c. GC/MS chromatogram (SIM-Single Ion Chromatogram) of an extract of treated soil sample (day 3, Banizoumbou).

required to give a signal-to-noise ratio of 5 : 1. For both detectors this limit was evaluated as 0·0001 mg kg^{-1} for fipronil and metabolites A, B and C. For metabolite D this limit was evaluated as 0.0001 mg kg^{-1} with ECD detection and 0·0005 mg kg^{-1} with MS detection.

3 RESULTS AND DISCUSSION

The evolution of fipronil and its metabolites in the 0–10-cm layer is presented in Figs. 3a and b for Banizoumbou and Saguia soils, respectively. Fipronil levels fell with time from 0·002 mg kg^{-1} to below the limit of quantification at Banizoumbou and from 0·001 to 0·0002 mg kg^{-1} at Saguia. Fipronil itself never leached beyond the 10-cm layer. In addition, on days 14 and 28, the 0–10-cm soil layer was divided into two (0–5 and 5–10 cm) and fipronil levels in the upper layer were noticeably higher than those in the 5–10-cm layer.

Degradation of fipronil in soil was evaluated concurrently from these same residue results. Overall, fipronil disappeared very quickly from the two soils. For the 0–10-cm soil layer at Banizoumbou, we observed a rapid fall in fipronil levels with an apparent half-life, determined by extrapolation from the curve presented in Fig. 3a, close to 36 h. Three days after treatment, fipronil had been 75% degraded and the four metabolites previously identified had appeared: metabolite A (dessulfinyl photodegradate), B (sulfide), C (sulfone) and D (amide). Of these only the amide migrated into the 10–20-cm layer (Fig. 4). The heavy rainfall (64 mm)

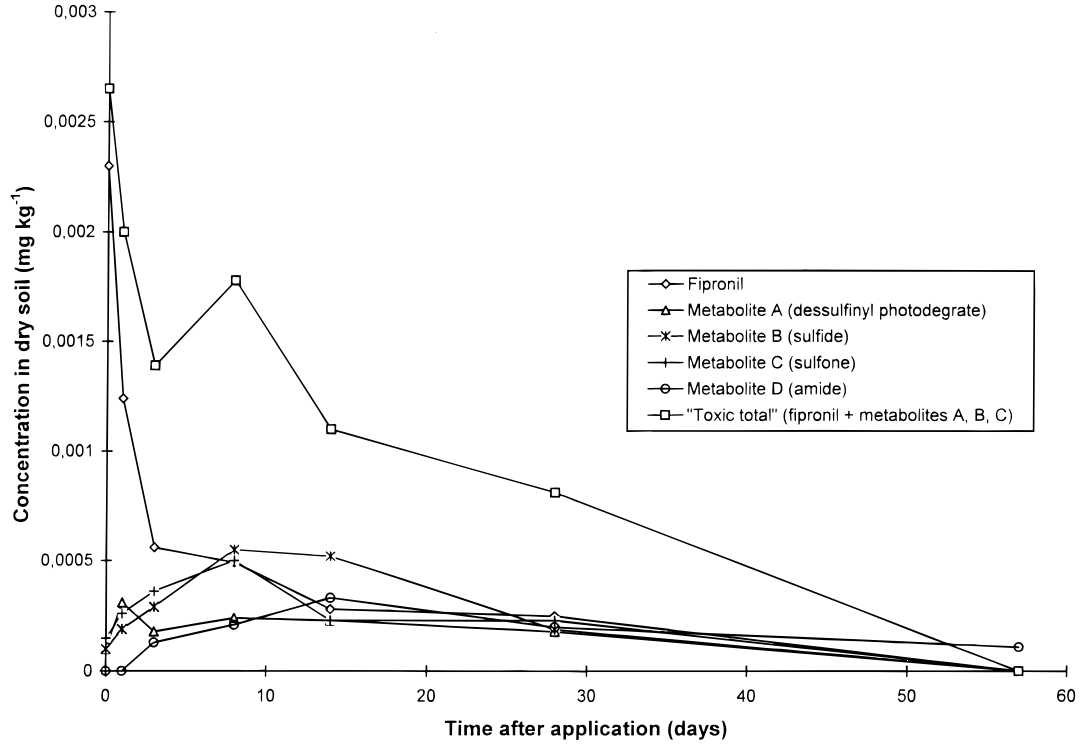

Fig. 3a. Behaviour of fipronil and its metabolites in a Sahelian soil (Banizoumbou, layer 0–10 cm).

occurring at Banizoumbou the day after treatment certainly contributed to the movement of this compound which is the most polar and water-soluble of the five. In Saguia soil, evaluation of the half-life of fipronil was made more difficult by the fact that the day 0 sampling was delayed until 8 h after treatment. This led to a noticeable fall in residue level for this first sampling, compared with Banizoumbou. However, the half-life remained close to that previously observed.

The same metabolites appeared, apart from the sulfide, which, although relatively abundant at Banizoumbou, was not formed in Saguia soil (Fig. 3b). After the third day, fipronil and metabolites A, C and D were degraded more slowly in Saguia than in Banizoumbou

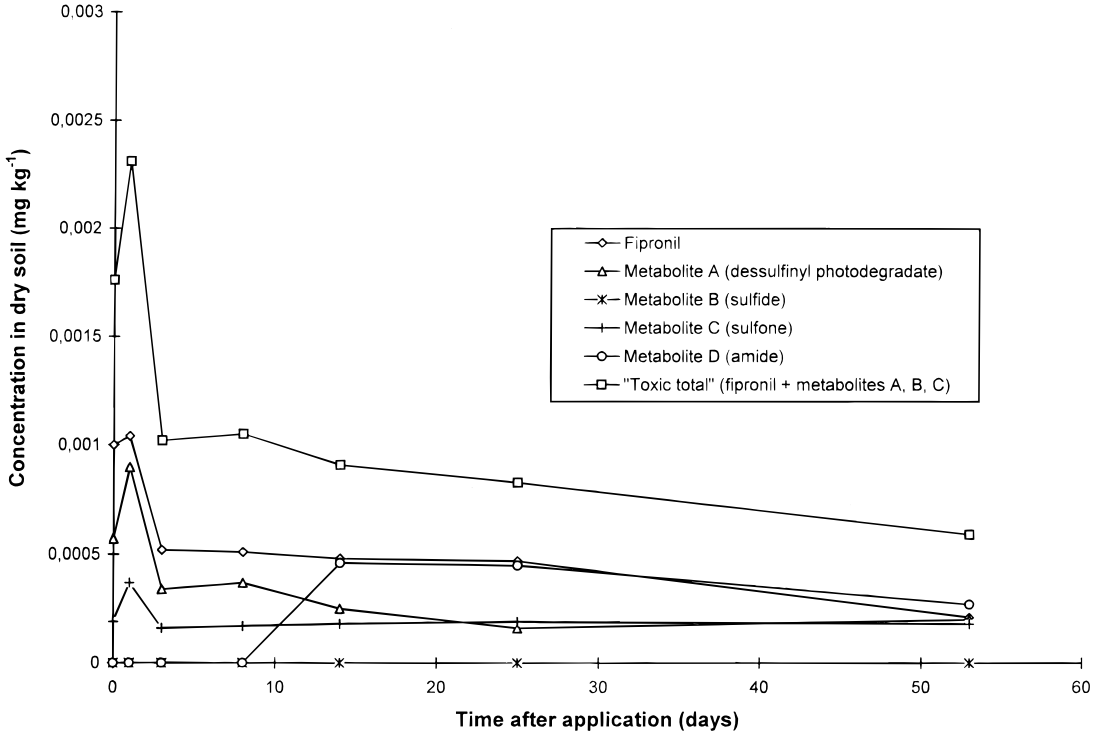

Fig. 3b. Behaviour of fipronil and its metabolites in a Sahelian soil (Saguia, layer 0–10 cm).

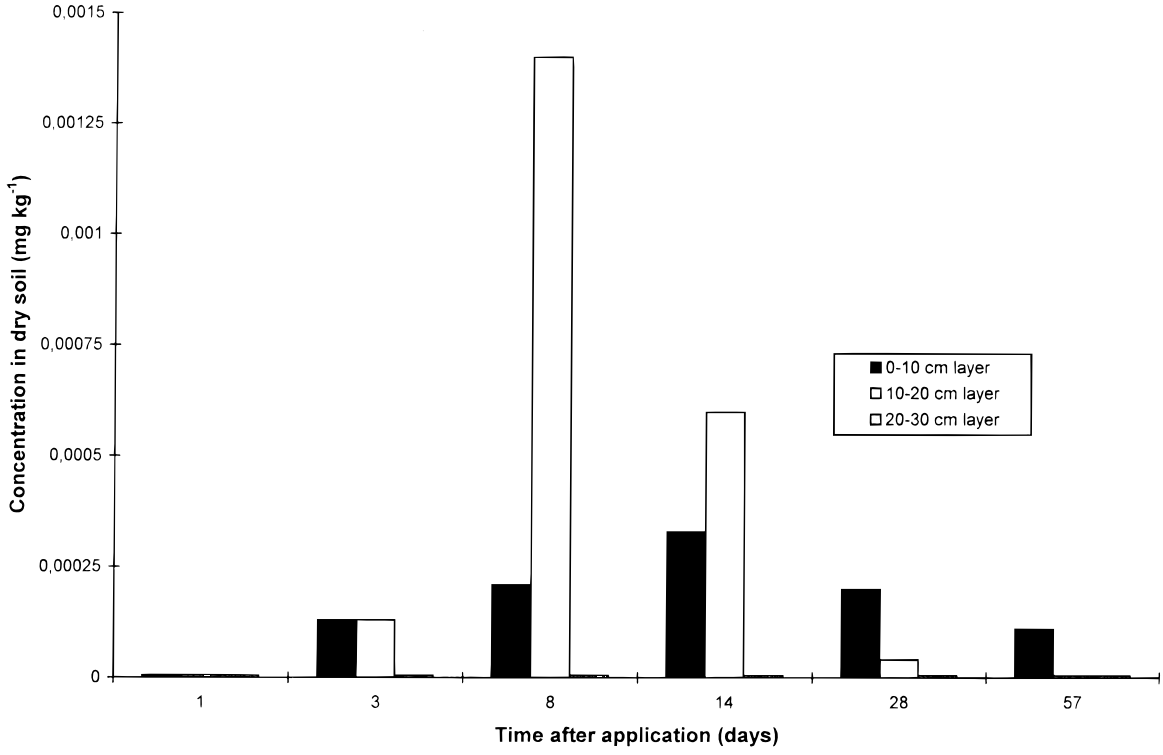

Fig. 4. Movement of metabolite D (amide) in Banizoumbou soil.

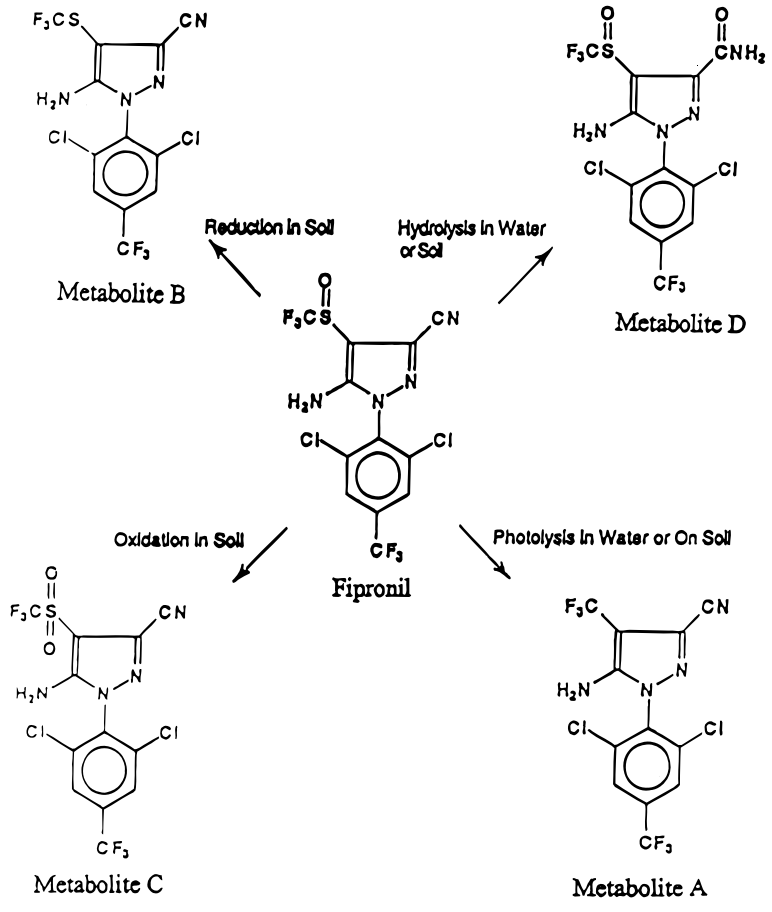

Fig. 5. Degradation pathways of fipronil in the environment.

soil. After rapid appearance of the photodegradate and sulfone during the first days (concomitant with the degradation of fipronil), the process seemed to stabilise, the compounds subsequently appearing only very slowly. In the same soil, the amide appeared only after day 8, which is noticeably later than at Banizoumbou, and did not pass into the 10–20-cm layer, probably due to the absence of the rainfall that occurred at Banizoumbou at this time.

Several studies[7,8] have confirmed that the efficacy of fipronil lasts for several weeks. The rapid degradation of fipronil reported here suggests that some of the metabolites also possess biological activity. Indeed, based on the results of in-vitro GABA-receptor assays and laboratory insect screening studies on indicator species, metabolites A, B and C are biologically active (Rhône-Poulenc Agro, 1995, pers. comm.). On the other hand, the amide is not considered to be a biologically active or toxicologically significant metabolite, since it is not toxic to rats at levels of 2000 $mg\,kg^{-1}$.[9] For this reason, we preferred to employ the concept of 'toxic total' corresponding to the sum of levels of fipronil and metabolites A, B and C. This 'toxic total' was also evaluated for each soil, in the 0–10-cm layer (Fig. 3a and b), thus enabling the persistence of action of the formulation to be explained.

The degradation pathways of fipronil, previously described by Rhône-Poulenc Agro, are presented in Fig. 5. Our results confirmed these pathways under plain field conditions:

Metabolite A is formed photochemically. Transformation is rapid when fipronil is not protected from the light by the soil, as was illustrated particularly in Saguia soil, where the degradation may be due to the higher exposure to sunshine on the day of treatment.

Metabolite B is formed by reduction and was found in Banizoumbou soil but not in Saguia soil. Although both soils are sandy, the higher level of silt in Banizoumbou and its relatively high moisture content may have meant that it was less aerated than the Saguia soil, thus favouring the reduction process.

Metabolite C is formed by oxidation. This metabolite has been found previously in a laboratory study (aerobic conditions) and under plain field conditions in a Spanish soil (Seville).

Metabolite D results from the hydrolysis of the nitrile group of fipronil to an amid group. The delay in the formation of the amide (Figs. 3a and b) leads us to suspect a biological rather than chemical hydrolysis. The microbial activity of Saguia soil, given its organic matter content, is certainly very low, which could explain the late appearance there of this metabolite and its very slow disappearance.

This study of the behaviour of fipronil under Sahelian conditions has demonstrated both a more rapid degradation than that observed in temperate climates, with a low leaching tendency for fipronil and its main metabolites.

ACKNOWLEDGEMENTS

The authors wish to thank Rhône-Poulenc Agro and Dr M-N. De Visschers and G. Balança (Cirad-Gerdat-Prifas) for their help throughout this study.

REFERENCES

1. Gant, D. B., Bloomquist, J. R., Ayad, H. M. & Chalmers, A. E., *Pestic. Sci.*, **30** (1990) 355–7.
2. Cole, L. M., Nicholson, R. A. & Casida, J. E., *Pestic. Biochem. Physiol.*, **46** (1993) 47–54.
3. Hosie, A. M., Baylis, H. A., Buckingham, S. D. & Sattelle, D. B., *Br. J. Pharmacol.*, **115** (1995) 909–12.
4. Bloomquist, J. R., *Annu. Rev. Entomol.*, **41** (1996) 163–90.
5. Rachadi, T., *Phytoma*, **474** (1995) 20–4.
6. F.A.O., Pesticide residue trials to provide data for the registration of pesticides and establishment of maximum residue limit. FAO, 1986.
7. Balança, G. & De Visscher, M. N., *Arch. Environ. Contam. Toxicol.*, **32** (1997) 58–62.
8. Balança, G. & De Visscher, M. N., *Crop Protection* (1997), in press.
9. Dange, M., RPA200766, Acute Oral LD_{50} in the Rat. Rhône-Poulenc Agro, internal report, Study No. SA93016, 1993.